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Gene Expression for a Novel Protein RGPR-p117 in Various Species: The Stimulation by Intracellular Signaling Factors

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Abstract The presence and expression for the gene encoding a novel regucalcin gene promoter region-related protein (RGPR-p117) in various species was investigated by using Southern ''zoo blot'' and Northern hybridization analyses. A ''zoo blot'' analysis demonstrated that RGPR-p117 gene was widely conserved in various species including human, rat, mouse, dog, cow, pig, rabbit, chicken, fish, *C. elegans* and yeast. The gene was not found in *Xenopus*. Northern blot analysis showed that RGPR-p117 mRNA was expressed in the liver of human, rat, mouse, and rabbit as a single mRNA of ~4.5 kb, respectively. However, homologous mRNA was not found in the liver of *Xenopus*. The expression of RGPR-p117 mRNA in liver was clearly enhanced 5 h after a single intraperitoneal administration of $CaCl_2$ (5 mg Ca^{2+}/100 g body weight) to rats. The RGPR-p117 mRNA is also expressed in the cloned H4-II-E rat hepatoma cells, although this expression was weak as compared with that of liver tissues. Moreover, the RGPR-p117 mRNA expression in H4-II-E cells was stimulated in the presence of dibutyryl cAMP, PMA, insulin, 17β-estradiol, or serum in culture medium. The present study demonstrates that the RGPR-p117 gene is conserved in various species, and that its expression is stimulated by intracellular signaling factors. J. Cell. Biochem. 87: 188–193, 2002.

Key words: RGPR-p117; regucalcin; gene expression; signaling factor

Regucalcin plays a pivotal role as a regulatory protein in calcium signaling [reviewed in Yamaguchi, 2000a,b]. A regucalcin gene promoter region-related protein (RGPR-p117) is a novel protein. This protein was found as a protein which can bind to TTGGC motif of regucalcin gene promoter region [Shimokawa and Yamaguchi, 1993; Murata and Yamaguchi, 1999; Misawa and Yamaguchi, 2000a,b; Misawa and Yamaguchi, 2001]. The entire rat RGPR-p117 cDNA consists of a 4,378 bp which contains an open reading frame (ORF) of 3,174 bp encoding a protein of 1,058 amino acid residues [Misawa and Yamaguchi, 2001]. RGPR-p117 cDNA is also identified in human and mouse [Misawa and Yamaguchi, 2001]. The amino acid sequence of rat RGPR-p117 had 70.9 and 88.5% homologies, as compared with that of human and mouse RGPR-p117, respectively [Misawa and Yamaguchi, 2001]. The comparison of human RGPR-p117 cDNA sequence with the genomic sequence database indicates that the gene is consisted of at least 26 exons spanning ~41 kb and localized on human chromosome 1q25.2 [Misawa and Yamaguchi, 2001]. This cDNA clone is highly identical to a front half part of the human RGPR-p117 cDNA, which is a splicing variant of human RGPR-p117 derived from human placental choriocarcinoma [Misawa and Yamaguchi, 2001].

The cellular function of RGPR-p117 is unknown. PROSITE search gives that RGPR-p117 has a leucine zipper motif $[L(X)_6L(X)_6L(X)_6L]$, and that its location is conserved among rat, mouse, and human [Landschulz et al., 1988; O'Shea et al., 1989a; Busch and Sassone-Corsi, 1990; Bairoch et al., 1997; Misawa and Yamaguchi, 2001]. The leucine

Abbreviations used: RGPR-p117, regucalcin gene promoter region-related protein; b(p), base(pair); FBS, fetal bovine serum; dibutyryl cAMP, N^6,2′-dibutyryl cyclic adenosine 3′,5′-monophoshate; PMA, phorbol 12-myristate 13-acetate; DIG, digoxigenin.

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zipper pattern is present in many gene regulatory proteins [Clerc et al., 1988; Maekawa et al., 1989; Vinson et al., 1989; O'Shea et al., 1989b; Collum and Alt, 1990]. It is speculated that RGPR-p117 may have a role as a gene regulatory protein.

The present study was undertaken to clarify the evolutionary conservation of RGPR-p117 gene and the expression of RGPR-p117 mRNA. We found that the RGPR-p117 gene is present in various species, and that the RGPR-p117 mRNA expression in the liver is enhanced by the administration of $CaCl_2$ to rats in vivo. Moreover, the RGPR-p117 mRNA was found to be expressed in the cloned rat hepatoma H4-II-E cells and the expression was stimulated in the presence of dibutyryl cAMP, insulin, PMA, 17β-estradiol, or serum in culture medium in vitro.

MATERIALS AND METHODS

Materials

Fetal bovine serum (FBS), N^6,2′-dibutyryl cyclic adenosine 3′,5′-monophoshate (dibutyryl cAMP), insulin, phorbol 12-myristate 13-acetate (PMA), and 17β-estradiol were purchased from Sigma (St. Louis, MO). S(−)-Bay K 8644 was obtained from Research Biochemicals International (Natick, MA). Deoxycytidine 5′-[α-^{32}P]triphosphate ([α-^{32}P]dCTP; 111 TBq/mmol) was purchased from New England Nuclear (Boston, MA). Molecular size standards (0.28–6.6 kb RNA ladder) for electrophoresis of RNA were purchased from Promega (Madison, WI). Digoxigenin (DIG) RNA labeling kit was obtained from Roche Molecular Biochemical, Inc. (Indianapolis, IN). Other reagents and chemicals used were of the highest grade of purity commercially available.

Southern Blot Analysis

A "zoo blot" was purchased from Seegene (Seoul, Korea). It contained 10 μg of *Eco* RI-digested genomic DNA from human, rat, mouse, dog, cow, pig, rabbit, chicken, frog (*Xenopus*), fish (Zebrafish), *C. elegans*, and yeast. The blot was prehybridized at 37°C for 4 h in a solution containing 50% formamide, 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. Hybridization was then performed for 18 h at 37°C with an [α-^{32}P]-labeled 1,007 bp *Eco* RV–*Hind* III fragment of rat RGPR p117 cDNA [Misawa and Yamaguchi, 2001]. The blot was washed twice for 5 min in 2 × SSC and 0.1% SDS at room temperature and then for 15 min in 0.5 × SSC and 0.1% SDS at room temperature, and then the blot was analyzed by a Fujix bio-image analyzer BAS2000 (Fuji Photo Film Co. Ltd., Japan).

Animals and Administration Procedure

Male Wistar rats, weighing 90–100 g, purchased from Japan SLC, Inc. (Hamamatsu, Japan), were fed commercial laboratory chow (solid, Oriental Yeast Company Ltd., Tokyo, Japan) containing 57.5% carbohydrate, 1.1% calcium, and 1.1% phosphorus, and distilled water, ad libitum. $CaCl_2$ was dissolved in sterile distilled water at a concentration of 10 mg of Ca^{2+}/ml. The solution (0.5 ml/100 g body weight) was intraperitoneally administered to rats [Murata and Yamaguchi, 1998]. Rats were sacrificed by bleeding at 0.5, 1, and 5 h after the administration. Control animals received vehicle solution. The liver was immediately removed from rats for the preparation of RNA.

Cell Culture

The cloned rat hepatoma H4-II-E cells were cultured as previously described [Murata and Yamaguchi, 1999]. The hepatoma cells (2.5×10^5) were maintained for 48 h in α-MEM supplemented with 5 mM glucose, 10% heat-inactivated FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin in humidified 5% CO_2/95% air at 37°C to obtain 70% confluent monolayers. After culture, the medium was changed, and maintained for 16 h in a serum-free α-MEM, and then cultured for 24 h in the same medium supplemented with or without 10 μM Bay K 8644, 0.1 mM dibutyryl cAMP, 100 nM insulin, 1 μM PMA, 10 nM 17β-estradiol, or 10% FBS before harvesting.

RNA Isolation

Total RNAs from liver (rat, mouse, rabbit, and *Xenopus*) and H4-II-E cells were prepared as described [Chomczynski and Sacchi, 1987]. Hepatoma cells or liver tissues were homogenized in buffer solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol, and 2 M sodium acetate. Total RNAs were extracted by vigorous shaking in a mixture of phenol, chloroform, and isoamyl alcohol, and phases were separated by centrifugation at 10,000*g* for 20 min at 4°C. RNA located in the

aqueous phase was precipitated with isopropanol at −20°C. RNA precipitates were pelleted by centrifugation, and the pellets were redissolved in 50 μl of DEPC-treated water. Human liver total RNA was purchased from Clontech (Palo Alto, CA).

Northern Blot Analysis

Ten micrograms of total RNAs extracted from liver tissues or hepatoma cells were electrophoresed in 1.2% agarose denaturing gels containing 2.2 M formaldehyde in MOPS buffer (pH 7, containing 20 mM 3-morpholinopropanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA) with 3 V/cm^3 for 3 h [Sambook et al., 1989]. The electrophoresed gels were transferred to nylon membranes by blotting. Northern blots were probed with DIG-labeled RGPR-p117 and G3PDH cRNA, respectively [Misawa and Yamaguchi, 2001]. The RGPR-p117 cRNA probe was corresponding to the position of 1,120–2,130 in rat RGPR-p117 cDNA antisense sequence [Misawa and Yamaguchi, 2001]. The blots were detected with alkaline phosphatase-conjugated anti-DIG antibody and a chemiluminescent substrate according to the manufacturer's instructions (Roche). The size of mRNA was determined using an RNA ladder (Promega).

Statistical Analysis

Data were expressed as the mean ± SEM. The significant of difference between the values was estimated by Student's *t*-test or by analysis of variance (ANOVA) for comparing multiple groups. A P value of <0.05 was considered to indicate a statistically significant difference.

RESULTS

Existence of RGPR-p117 Gene in Various Species

A "zoo blot" analysis was performed to determine the presence of RGPR-p117 gene in various species. Southern blots with human, rat, mouse, dog, cow, pig, rabbit, chicken, frog (*Xenopus*), fish (Zebrafish), *C. elegans*, and yeast genomic DNA were hybridized with the 1,007 bp *Eco* RV–*Hind* III fragment of rat RGPR-p117 cDNA [Misawa and Yamaguchi, 2001] (Fig. 1). The blot showed that the RGPR-p117 gene was found in all mammalian, avian, and fish species used, but the band of gene was only slightly in frog. Clear bands were found in

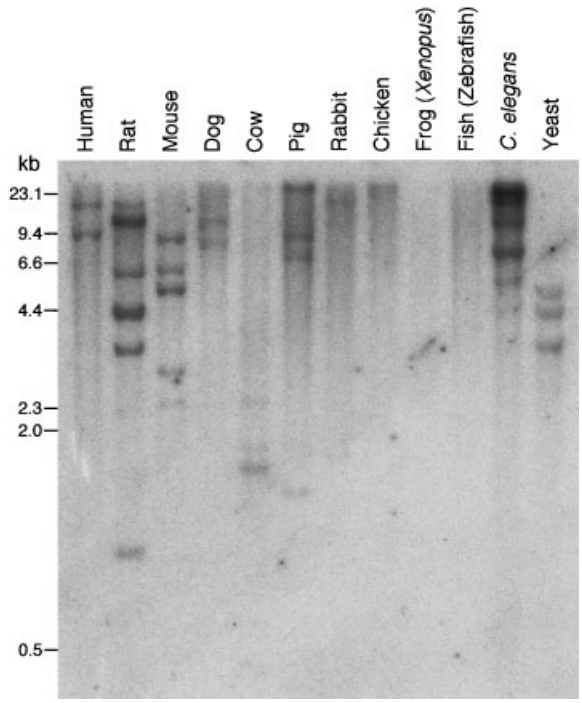

Fig. 1. Genomic Southern hybridization analysis of the RGPR-p117 gene. The "zoo blot" was hybridized with a ^{32}P-labeled 1.0 kb fragment excised from rat RGPR-p117 cDNA by *Eco* RV and *Hind* III digestion. Each lane contained 10 μg of the *Eco* RI digested genomic DNA from human, rat, mouse, dog, cow, pig, rabbit, chicken, frog (*Xenopus*), fish (Zebrafish), *C. elegans*, and yeast. The figure shows one of three experiments.

C. elegans and yeast genomic DNA, indicating that the genes with homologies to the rat RGPR-p117 gene were present in their species.

The expression of RGPR-p117 mRNA has been shown to be present in the liver, kidney, heart, spleen, and brain of rats [Misawa and Yamaguchi, 2001]. RGPR-p117 mRNA expression in the liver of various animal species is shown in Figure 2. Northern blot analysis with DIG-labeled rat RGPR-p117 cRNA probe showed that RGPR-p117 mRNA was expressed in the liver of human, rat, mouse, and rabbit as a single mRNA with ~4.5 kb, respectively. However, the expression of homologous mRNA was not clearly found in the liver of frog. These results indicate a high degree of evolutionary conservation of the RGPR-p117 gene.

Enhancement of the RGPR-p117 mRNA Expression in Rat Liver by Ca^{2+} Administration

The effect of calcium administration on RGPR-p117 mRNA levels in the liver of rats is shown in Figure 3. Rats were sacrificed 0.5, 1, or 5 h after a single intraperitoneal administration of $CaCl_2$ (5 mg of Ca^{2+}/100 g body weight). Total

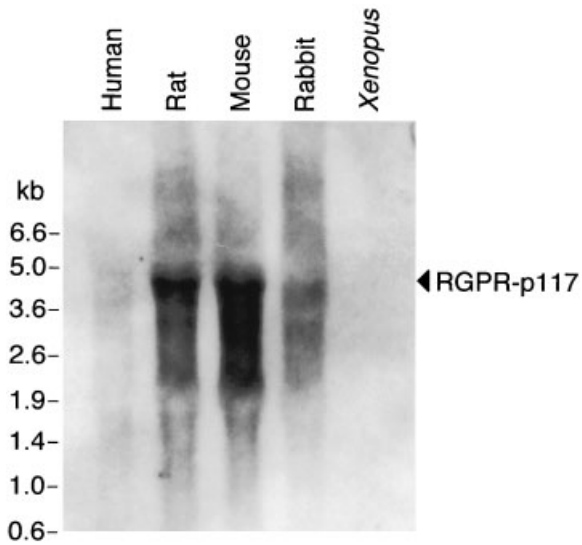

Fig. 2. Northern blot analysis of RGPR-p117 mRNA expression in the liver of different animals. The total RNAs (10 μg) extracted from human, rat, mouse, rabbit, and *Xenopus* livers were subjected to Northern blot analysis. The blot was hybridized to DIG labeled RGPR-p117 probe. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of three experiments.

RNAs from the liver were prepared, and 10 μg of total RNAs were subjected to the Northern blot analysis. Liver RGPR-p117 mRNA levels were not changed at 0.5 and 1 h after the administration of $CaCl_2$. RGPR-p117 mRNA expression was significantly increased at 5 h after the administration.

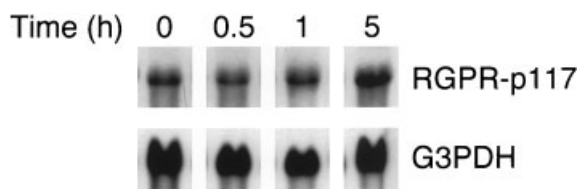

Fig. 3. Effect of calcium administration on the RGPR-p117 mRNA expression in the liver of rats in vivo. Rats received a single intraperitoneal administration of $CaCl_2$ (5 mg of Ca^{2+}/100 g body weight), and 0.5, 1, or 5 h later the animals were killed by bleeding. Total RNAs from liver were prepared immediately. Control rats received an equivalent volume of distilled water. Ten micrograms of total RNAs were subjected to the Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**upper panel**) or G3PDH (**lower panel**) cRNA, respectively. The densitometric data of liver RGPR-p117 mRNA levels at 0.5, 1, or 5 h after the administration of $CaCl_2$ were 98.7 ± 2.3, 104.7 ± 1.8, and 169.0 ± 2.1 (% of control; mean ± SEM of five rats), respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of five experiments with separate rats. The data obtained at 5 h after the administration were significant ($P < 0.01$) as compared with the control value.

Expression of the RGPR-p117 mRNA in the Cloned Rat Hepatoma H4-II-E Cells

The expression of RGPR-p117 mRNA in the cloned rat hepatoma H4-II-E cells is shown in Figure 4. RGPR-p117 mRNA in the hepatoma cells was found as a single band with ~4.5 kb as found in liver tissues. RGPR-p117 mRNA levels in the hepatoma cells were lowered as compared with that of rat liver tissues.

Effect of Signaling Factors on the RGPR-p117 mRNA Expression in the Cloned Rat Hepatoma H4-II-E Cells

The hepatoma cells were cultured in the presence of either vehicle (control), 10 μM Bay K 8644, 0.1 mM dibutyryl cAMP, 1 μM PMA, 100 nM insulin, 10 nM 17β-estradiol, or 10% FBS. Total RNAs prepared from the cells were subjected to the Northern blot analysis (Fig. 5). The expression of RGPR-p117 mRNA was clearly increased in the presence of dibutyryl cAMP, insulin, PMA, 17β-estradiol, or serum. However, the effect of Bay K 8644 was slight.

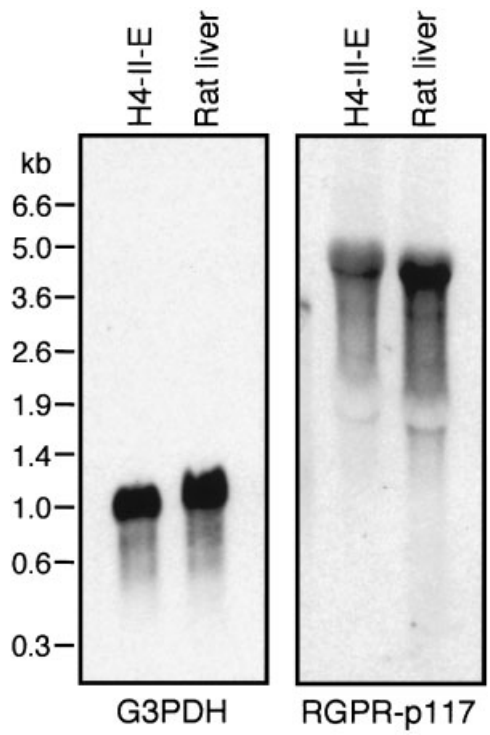

Fig. 4. The expression of the RGPR-p117 mRNA in the cloned rat hepatoma H4-II-E cells. Ten micrograms of total RNAs were subjected to the Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**right panel**) or G3PDH (**left panel**) cRNA, respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of four experiments.

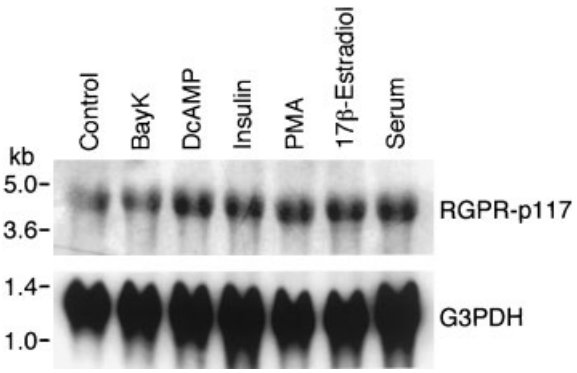

Fig. 5. Effect of various intracellular signaling factors on the RGPR-p117 mRNA expression in the cloned rat hepatoma H4-II-E cells. The cells with subconfluent cultured for 24 h in a medium containing either vehicle (control), 10 μM Bay K 8644, 0.1 mM dibutyryl cAMP, 100 nM insulin, 1 μM PMA, 10 nM 17β-estradiol, or 10% FBS. The cells were harvested and prepared total RNAs. Ten micrograms of total RNAs were subjected to the Northern blot analysis. Blots were hybridized with the probes of RGPR-p117 (**upper panel**) or G3PDH (**lower panel**) cRNA, respectively. The densitometric data of RGPR-p117 mRNA levels in the presence of Bay K 8644, dibutyryl cAMP, insulin, PMA, 17β-estradiol, and FBS were 105.0 ± 2.3, 154.0 ± 2.6, 138.0 ± 4.4, 134.7 ± 3.3, 141.7 ± 4.4, and 153.7 ± 3.2 (% of control; mean ± SEM of four experiments), respectively. The positions of the markers are indicated alongside the RNA lanes. The figure shows one of four experiments with separate cell culture. The data from dibutyryl cAMP, insulin, PMA, 17β-estradiol, and FBS were significant ($P < 0.01$) as compared with the control value.

DISCUSSION

Regucalcin has been to be demonstrated to play a pivotal role as a regulatory protein of Ca^{2+} signaling [Yamaguchi, 2000a,b]. A novel protein RGPR-p117 has been found as the RGPR-p117 from rat liver [Misawa and Yamaguchi, 2001]. RGPR-p117 is seemed to be a transcriptional factor, which is involved in regucalcin gene expression [Misawa and Yamaguchi, 2001]. RGPR-p117 cDNA is also isolated from the liver of human and mouse [Misawa and Yamaguchi, 2001]. The present study shows that RGPR-p117 gene is present in mammalian and avian species with the "zoo blot" analysis, suggesting that the gene is evolutionally conserved in many species. The conservation of RGPR-p117 gene in mammals was also seen in the expression of their mRNA in the liver of human, rat, mouse, and rabbit.

The expression of RGPR-p117 mRNA was found to be difference with various species. Rat and mouse RGPR-p117 mRNAs in the liver were clearly detected, while rabbit RGPR-p117 mRNA was comparatively low level. Human liver RGPR-p117 mRNA was detected as a faint band. The nucleotide sequence of the *Eco* RV–*Hind* III fragment of rat RGPR p117 cDNA used as probe was homologies of 95 and 90% for the corresponding region of mouse and human RGPR-p117 cDNAs, respectively. This result suggests that rabbit RGPR-p117 gene is more related to the rodent gene than that of human. Frog or fish RGPR-p117 gene was only slightly detected with both "zoo blot" and Northern blot analyses, suggesting that RGPR-p117 gene has less homology in frog or fish. However, a clear band of fish RGPR-p117 gene was observed under highly hybridization temperature (42°C) (data not shown), indicating that RGPR-p117 gene exists in fish. Meanwhile, the clear bands were detected in *C. elegans* and yeast genomic DNA using the rat cDNA probe, indicating that the genes with homologies to the rat RGPR-p117 gene are present in their species.

The expression of RGPR-p117 mRNA in the liver was found to be stimulated by calcium administration to rats in vivo. The effect of calcium administration in stimulating the RGPR-p117 mRNA expression in rat liver was seen at late time (5 h) after the administration. This finding suggests that the liver RGPR-p117 mRNA expression is partly stimulated through the calcium administration-induced secondary factor(s) rather than a direct effect of calcium on the gene expression.

The expression of RGPR-p117 mRNA in the cloned rat hepatoma H4-II-E cells in vitro was examined. The RGPR-p117 mRNA was clearly expressed in the hepatoma cells, although its expression level was lower than that of rat liver tissues. RGPR-p117 mRNA in the hepatoma cells was detected as a single band of ~4.5 kb seen in rat liver, indicating that the H4-II-E cells may be a good tool to explore a possible regulatory mechanism of RGPR-p117 mRNA expression.

The expression of RGPR-p117 mRNA in the cloned rat hepatoma H4-II-E cells was found to be stimulated by the addition of serum (10% FBS) in culture medium, suggesting that the gene expression is regulated by signaling factors in the serum. The RGPR-p117 mRNA expression was significantly increased in the presence of dibutyryl cAMP, PMA, insulin, or 17β-estradiol in culture medium without serum addition. This finding demonstrates that RGPR-p117 mRNA expression in the hepatoma cells is regulated by various signaling factors.

The expression of regucalcin mRNA is shown to be stimulated by dibutyryl cAMP, Bay K 8644, PMA, or insulin in the cloned rat hepatoma cells [Yamaguchi and Nakajima, 1999]. Hepatic regucalcin mRNA expression has also shown to be stimulated by the administration of 17β-estradiol to rats [Yamaguchi and Oishi, 1995]. Presumably, RGPR-p117 is a transcriptional factor, which is involved in the stimulation of regucalcin gene expression in liver cells.

PMA is an activator for protein kinase C [Nishizuka, 1986]. Dibutyryl cAMP may be able to activate cyclic AMP-dependent protein kinase. The action of insulin is mediated through protein tyrosine kinase [Kido et al., 2001]. Presumably, the expression of RGPR-p117 mRNA is partly stimulated by signaling mechanisms which are mediated through various protein kinases. Moreover, RGPR-p117 mRNA expression may be partly stimulated through the receptors of 17β-estradiol which is localized in the cell nucleus.

The present finding, that RGPR-p117 mRNA expression is stimulated by intracellular signaling factors, suggests that RGPR-p117 protein may have a role in signaling system of liver cells. In addition, RGPR-p117 mRNA has been demonstrated to be expressed in the kidney, heart, spleen, and brain of rats [Misawa and Yamaguchi, 2001]. RGPR-p117 is expressed in many tissues. The role of a novel protein RGPR-p117 in their tissues is unknown, at present. However, RGPR-p117 has a leucine zipper motif which is conserved among RGPR-p117 in rat, mouse, and human [Misawa and Yamaguchi, 2001]. It is speculated that RGPR-p117 may be a transcriptional factor (or co-factor) for gene expression which is conserved with evolution.

In conclusion, it has been demonstrated that RGPR-p117 gene is highly conserved in various species, and that the gene expression is regulated by various signaling factors.

REFERENCES

Bairoch A, Bucher P, Hofmann K. 1997. The PROSITE database, its status in 1997. Nucleic Acids Res 25:217–221.

Busch SJ, Sassone-Corsi P. 1990. Dimers, leucine zippers, and DNA-binding domains. Trends Genet 6:36–40.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol–chloroform extraction. Anal Biochem 162:156–159.

Clerc RG, Corcoran LM, LeBowitz JH, Baltimore D, Sharp PA. 1988. The B-cell-specific Oct-2 protein contains POU box- and homeo box-type domains. Genes Dev 2:1570–1581.

Collum RG, Alt FW. 1990. Are myc proteins transcription factors? Cancer Cells 2:69–75.

Kido Y, Nakae J, Accili D. 2001. Clinical review 125: The insulin receptor and its cellular targets. J Clin Endocrinol Metab 86:972–979.

Landschulz WH, Johnson PF, McKnight SL. 1988. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. Science 240:1759–1764.

Maekawa T, Sakura H, Kanei-Ishii C, Sudo T, Yoshimura T, Fujisawa J, Yoshida M, Ishii S. 1989. Leucine zipper structure of the protein CRE-BP1 binding to the cyclic AMP response element in brain. EMBO J 8:2023–2028.

Misawa H, Yamaguchi M. 2000a. Involvement of hepatic nuclear factor I binding motif in transcriptional regulation of Ca^{2+}-binding protein regucalcin gene. Biochem Biophys Res Commun 269:270–278.

Misawa H, Yamaguchi M. 2000b. Intracellular signaling factors—enhanced hepatic nuclear protein binding to TTGGC sequence in the rat regucalcin gene promoter: Involvement of protein phosphorylation. Biochem Biophys Res Commun 279:275–281.

Misawa H, Yamaguchi M. 2001. Molecular cloning and sequencing of the cDNA coding for a novel regucalcin gene promoter region-related protein in rat, mouse, and human liver. Int J Mol Med 8:513–520.

Murata T, Yamaguchi M. 1998. Ca^{2+} administration stimulates the binding of AP-1 factor to the 5′-flanking region of the rat gene for the Ca^{2+}-binding protein regucalcin. Biochem J 329:157–163.

Murata T, Yamaguchi M. 1999. Promoter characterization of the rat gene for Ca^{2+}-binding protein regucalcin. Transcriptional regulation by signaling factors. J Biol Chem 274:1277–1285.

Nishizuka Y. 1986. Studies and perspectives of protein kinase C. Science 233:305–312.

O'Shea EK, Rutkowski R, Kim PS. 1989a. Evidence that the leucine zipper is a coiled coil. Science 243:538–542.

O'Shea EK, Rutkowski R, Stafford WF III, Kim PS. 1989b. Preferential heterodimer formation by isolated leucine zippers from fos and jun. Science 245:646–648.

Sambook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: A laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press. pp 739–752.

Shimokawa N, Yamaguchi M. 1993. Molecular cloning and sequencing of the cDNA coding for a calcium-binding protein regucalcin from rat liver. FEBS 327:251–255.

Vinson CR, Sigler PB, McKnight SL. 1989. Scissors-grip model for DNA recognition by a family of leucine zipper proteins. Science 246:911–916.

Yamaguchi M. 2000a. Role of regucalcin in calcium signaling. Life Sci 66:1769–1780.

Yamaguchi M. 2000b. The role of regucalcin in nuclear regulation of regenerating liver. Biochem Biophys Res Commun 276:1–6.

Yamaguchi M, Nakajima M. 1999. Involvement of intracellular signaling factors in the serum-enhanced Ca^{2+}-binding protein regucalcin mRNA expression in the cloned rat hepatoma cells (H4-II-E). J Cell Biochem 74:81–89.

Yamaguchi M, Oishi K. 1995. 17β-Estradiol stimulates the expression of hepatic calcium-binding protein regucalcin mRNA in rats. Mol Cell Biochem 143:137–141.